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Excretory secretory antigens of *Setaria cervi* microfilariae*

N. SRIVASTAVA, H. MUSTAFA, D. C. KAUSHAL[1], N. A. KAUSHAL**

Division of Biochemistry and [1]Division of Microbiology, Central Drug Research Institute, Lucknow-226 001, India

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Summary

The excretory-secretory (ES) products from microfilariae (Mf) of *Setaria cervi*, a bovine filarial parasite, were prepared by *in vitro* maintenance of microfilariae at 37°C in Ringer's solution. The microfilarial ES (MfES) products were analysed by immunoelectrophoresis, counter-current immunoelectrophoresis and enzyme-linked immunosorbent assay (ELISA) using immune rabbit sera. Immunoelectrophoretic analysis of MfES products using rabbit anti-*S. cervi* Mf antigen serum, showed the presence of 6—7 antigens in ES products while 3—4 and 4—5 antigens were observed with rabbit anti-*S. cervi* adult ES (ScA ES) serum and rabbit anti-*Brugia malayi* Mf (BmMf) serum, respectively. Both the immune rabbit sera (rabbit anti-ScMf and rabbit anti-BmMf) exhibited a reciprocal antibody titre of 400 000 in ELISA. The *S. cervi* MfES products showed high reactivity with the antibodies present in filarial patient sera and may provide an easily available source of diagnostic filarial antigens.

Key words: Filariasis; *Setaria cervi*; microfilariae; excretory secretory products; immunodiagnosis

Introduction

Filariasis is a serious health hazard affecting millions of people, all over the world, mostly living in the tropical and sub-tropical countries. The practical difficulties associated with the parasitological examination of the night blood smears have created interest to develop alternate methods for diagnosing filarial infections. The accurate and specific diagnosis of infection, at an early stage, is essential for effective management of the disease. The immunodiagnosis of filariasis has been the major challenge in the area of filaria immunology and the antigens released by the living parasites are shown to be the better source of diagnostic antigens (De S a v i g n y and T i z a r d, 1977). The diagnostic utility of filarial excretory-secretory (ES) antigens has been demonstrated earlier by several investigators both in case of lymphatic filariasis and onchocerciasis (K a u s h a l *et al.*, 1982, 1984; O t t e s e n, 1984). The ES antigens obtained from *Wuchereria bancrofti* microfilariae, the most prevalent species of human filarial parasite in India, have been successfully used by H a r i n a t h and co-worker (1986) for the immunodiagnosis of human filariasis. However, the major limitation is to obtain a constant supply of ES antigens from the human filarial parasites for field application. This has emphasised the need for isolating the antigens from alternate sources such as animal filarial parasites including *Setaria cervi*, a bovine filarial parasite.

In the present study, the excretory-secretory products released by the *S. cervi* microfilariae have been analysed using certain immunological techniques and evaluated for their immunodiagnostic potential.

Material and Methods

Motile adult worms of *Setaria cervi* were collected from the peritoneal folds of freshly slaughtered Indian water buffaloes in a local abattoir and brought to the laboratory in the normal saline. The worms were washed extensively with normal saline before being used for isolating the microfilariae. The microfilariae were obtained by dissecting longitudinally the adult females of *S. cervi* and incubating the distal portions of uteri in Ringer's solution (S i n g h a l *et al.*, 1973) at 37°C. The medium was centrifuged at 800 x g and the microfilariae obtained were used for the preparation of excretory-secretory (ES) products.

Microfilariae (Mf) were maintained in Ringer's solution at 37°C. The medium was changed after each hour and the incubation was continued up to 6 hour. MfES medium was collected by centrifugation and concentrated

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** Corresponding author

by lyophilisation. The concentrated medium was used as source of MfES product for further analysis. Protein content of MfES products was determined by the colorimetric method of Lowry *et al.* (1951).

The immune rabbit sera against *S. cervi* Mf somatic antigen (Malhotra *et al.*, 1986) *S. cervi* adult ES (Malhotra *et al.*, 1987) and *Brugia malayi* Mf somatic antigen (Kaushal *et al.*, 1987), already available in the laboratory, were used in the study. Patient sera were collected from the individuals having filarial infection. Normal human sera were collected from normal healthy individuals from a non-endemic area.

Immunoelectrophoresis was carried out according to the method of Malhotra *et al.* (1987). *S. cervi* MfES products were separated in 1.5 mm thick layer of 1% (w/v) agarose for 1 hour at 150 V. After electrophoresis, antigen was allowed to react with antiserum by incubating for 18—20 h in a moist chamber. The plate was washed thrice with normal saline, pressed under several sheets of filter paper. The plate was air dried, stained with Coomassie blue (0.25%) and destained.

Counter-current immunoelectrophoresis (CIEP) was done according to method of Fossieck *et al.* (1973) MfES products and antisera were applied in the wells separately and the electrophoresis was run for 30 minutes at constant voltage (150 V). After that, the plate was washed, air dried and stained as described for IEP.

ELISA was performed according to the method of Voller *et al.* (1974). The wells of microtitre plate were coated with *S. cervi* MfES products (0.1 µg/well) by keeping the plate overnight at 37°C. Plate was washed with PBS-Tween (Phosphate-Buffered saline containing 0.05% Tween-20) and incubated with 2% milk for 2 h at 37°C in order to block the uncoated sites. After blocking, the plate was incubated with different dilutions of immune sera for 2 h followed by incubation with anti-rabbit peroxidase conjugate for 1 1/2 h. In each step, the plate was washed with PBS-Tween. The plate was finally developed with substrate solution (1 mg/ml orthophenylenediamine in citrate buffer, pH 5.4, containing 1 µl/ml hydrogen peroxide). The reaction was stopped with 5N H_2SO_4 and the intensity of colour was recorded in ELISA-Reader at 490 nm.

Results

The *S.cervi* microfilariae, when maintained *in vitro* in Ringer's solution, released appreciable amount of protein (8.8 mg/g wet weight of mf) in the medium over a period of 6 h. The conditions for the collection of *S. cervi* MfES products were optimised by following the hourly release of ES products. The Mf released approximately 1.35 mg protein/g wet wt/hour during the *in vitro* incubation and remained motile up to 5 hours after that decrease in the motility and release of ES products was observed. Therefore, in all subsequent experiment, 5 h incubation period was used for the collection of MfES products.

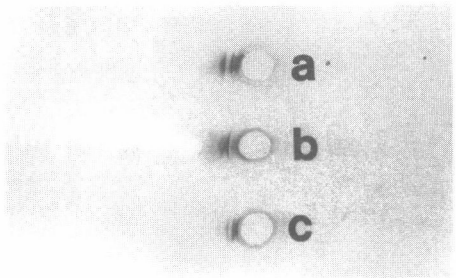

Fig. 1. Counter-current immunoelectrophoresis of *Setaria cervi* MfES products using rabbit anti-ScMf serum (a), rabbit anti-ScAES serum (b) and rabbit anti-BmMf serum (c)

The antigenic analysis of *S. cervi* MfES products was done by immunoelectrophoretic techniques and ELISA

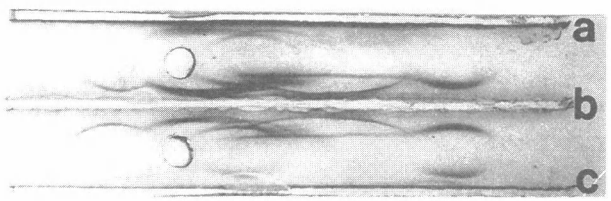

Fig.2. Immunoelectrophoretic analysis of *Setaria cervi* MfES products using rabbit anti-BmMf serum (a), rabbit anti-ScMf serum (b) and rabbit anti-ScAES serum (c)

using the immune rabbit sera. The counter-current immunoelectrophoretic and immunoelectrophoretic patterns of *S. cervi* MfES are shown in Fig. 1 and 2. Five, three and four precipitin bands were observed in *S. cervi* MfES products with rabbit anti-*S. cervi* Mf serum (rabbit anti-ScMf), rabbit anti-*S.cervi* adult ES serum (rabbit anti-ScAES) and rabbit anti-*B. malayi* Mf serum (rabbit anti-BmMf) respectively in CIEP (Fig. 1, Tab. 1). The immunoelectrophoretic analysis revealed 6—7, 3—4 and

Tab. 1. Counter-current immunoelectrophoresis, immunoelectro phoresis and ELISA of *S. cervi* MfES with hyperimmune rabbit sera

Sera	Number of bands in CIEP	Number of bands in IEP	Reciprocal ELISA titres
Rabbit anti-ScMf serum	5	6—7	400 000
Rabbit anti-adult ScES serum	3	3—4	48 600
Rabbit anti-BmMf	4	4—5	400 000

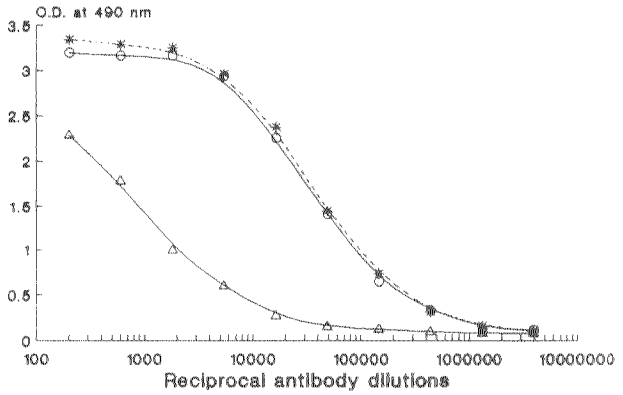

Fig. 3. Titration of rabbit anti-ScMf serum (O), rabbit anti-ScAES serum (Δ) and rabbit anti-BmMf serum (✱) against *Setaria cervi* MfES products in ELISA

4—5 antigens in MfES products with rabbit anti-ScMf, rabbit anti-ScAES and rabbit anti-BmMf serum respectively (Fig. 2, Tab. 1).

The antigenicity of *S. cervi* MfES products was further confirmed by testing the reactivity of *S. cervi* MfES products in ELISA using the immune rabbit sera. The titration curves obtained with the three immune rabbit sera (rabbit anti-ScMf serum, rabbit anti-ScAES serum and rabbit anti-BmMf serum) are shown in Fig. 3. The rabbit anti-ScMf serum and rabbit anti-BmMf serum showed reciprocal antibody titres of 400 000 with *S. cervi* MfES products while a reciprocal antibody titre of 48 600 was obtained for rabbit anti-ScAES serum (Tab. 1).

Tab. 2. Reactivity of *S. cervi* MfES products with filarial patient sera in ELISA

Patient sera	OD 490 nm at 1:100 dilution	Titre value
Filarial patient sera		
1	2.695	1:1600
2	2.759	1:3200
3	2.877	1:6400
4	1.558	1:1600
5	1.377	1:1600
6	1.923	1:1600
7	3.620	1:12 800
8	3.235	1:6400
9	2.790	1:2000
Filarial patient pool	2.837	1:3200
Normal human sera		
1	0.332	—
2	0.229	—
3	0.225	—
4	0.201	—
5	0.312	—
Normal human serum pool	0.275	—

In order to determine whether the ES products obtained from *S. cervi* Mf were immunologically recognised by the antibodies in filariasis patients, ELISA was done using a pool of filariasis patient sera as well as few individual filarial patient sera. Normal human sera were also used as control. All the filariasis patients sera had significant levels of antibodies to *S.cervi* MfES products as shown by the titre values of these sera (1:1 600—1:12 800) and the OD 490 values at 1:100 dilution of these sera (Tab. 2).

Discussion

Excretory-secretory (ES) antigens released by the living parasite in the host, are known to be less complex and more specific in diagnosing parasitic infections (De S a v i g n y and T i z a r d, 1977; K a u s h a l *et al.*, 1982, 1984; O t t e s e n, 1984). ES antigens from *Wuchereria bancrofti* Mf have been used for antibody detection in filariasis (H a r i n a t h, 1986) but there is a limitation as one has to depend on infected individuals for obtaining the parasitic material due to the non-availability of suitable animal model for *W. bancrofti*. Though, the other human filarial parasite *B. malayi* could be maintained in animal models, this parasite released ES antigens in minute quantities as shown by earlier studies of K a u s h a l *et al.* (1982). Therefore, we have explored the possibility of using *Setaria cervi*, a bovine filarial parasite, for isolating the ES products. Common antigens between this bovine and human filarial parasites have been identified, in earlier studies from our laboratory, using the polyclonal (K a u s h a l *et al.*, 1987) and monoclonal antibodies (K a u s h a l *et al.*, 1994).

In the present study, we have analysed the ES products prepared from *S. cervi* microfilariae. The *S. cervi* Mf released almost the same amount of protein per hour and could be maintained upto 6 hours, after that they became sluggish. Therefore, in all further experiments, a 5 h incubation period was used for the collection of MfES products. Appreciable amount of ES products could be obtained from *S. cervi* Mf, in the present study, like that of *S. cervi* adults (M a l h o t r a *et al.*, 1987) whereas *B. malayi* adults have been shown to release ES products in microgram quantities (K a u s h a l *et al.*, 1982).

The antigenic analysis of *S. cervi* MfES by immunoelectrophoretic techniques revealed less complex pattern of *S. cervi* MfES products in comparison to *S. cervi* adult

ES product (M a l h o t r a *et al.*, 1987) and *B. malayi* ES products (K a u s h a l *et al.*, 1982) where 10—15 antigens were observed. Out of the 6—7 antigens observed in *S. cervi* MfES products, 4—5 were found to be common with *B. malayi* microfilaria and 3—4 common with *S. cervi* adult ES products. This was further shown by the reactivity of MfES products with the rabbit anti-ScAES serum, rabbit anti-BmMf serum and *W. bancrofti* infected patient serum pool in ELISA.

The MfES products showed high reactivity with the antibodies present in filarial patients sera there by suggesting the immunogenic nature of *S. cervi* MfES products. The ES products obtained from adult stage of this parasites have also shown high reactivity with the antibodies in filarial patients sera (M a l h o t r a, 1988). The reactivity of *S. digitata* (another bovine filarial parasite) adult somatic and adult ES antigens with filarial antibodies has been demonstrated earlier by other investigators (D i s s a n a y a k e and I s m a i l, 1980; S u g u n a n and R a j, 1990).

Therefore, in the present study, we have been able to prepare and analyse the ES products from *S. cervi* Mf. The MfES products appeared to be simpler, share antigenic epitopes with human filarial parasites and were found to be highly reactive with the antibodies induced during natural filarial infection. These findings suggest that *S. cervi* MfES may provide a cheap and easily available source of filarial antigens for diagnostic purposes.

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